7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring tin and its compounds, its metabolites, and other biomarkers of exposure and effect to tin and its compounds. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

Tin is usually determined as the total metal, but it may also be measured as specific organotin compounds. Flame atomic absorption analysis is the most widely used and straightforward method for determining tin; furnace atomic absorption analysis is used for very low analyte levels and inductively coupled plasma atomic emission analysis is used for multianalyte analyses that include tin.

The preferred separation technique for organotin compounds is gas chromatography (GC) due to its high resolution and detector versatility. Analysis of organotin compounds usually consists of four steps: (1) extraction; (2) formation of volatile derivative; (3) separation; and (4) detection and quantification. First, the organotin compounds must be extracted from the sample using organic solvents, ion exchange resins, or adsorption onto a solid support. For biological materials, a general clean-up step is needed, such as purification using Florisil, silica gel, alumina, or ion exchange resin. The extracted organotin compounds must then undergo derivatization to a volatile form to be able to separate them by GC. Derivatization methods include the formation of alkyl (methyl or pentyl) derivatives using a Grinard reagent, formation of ethyl derivatives using sodium tetraethylborate, or by formation of hydrides (R_nSnH_{4-n}) using sodium borohydride. Separation of these derivatives may be done using differences in their boiling points or by GC. Finally, detection and quantification can be performed using a flame photometric detector, atomic absorption spectroscopy (AAS), or mass spectrometry (MS) (Takeuchi et al. 2000; WHO 1990).

High performance liquid chromatography (HPLC) has also been used in the analysis of organotin compounds. The advantage of HPLC over GC is that no derivativization step is needed after extraction.

Most separations are based on ion exchange or reversed phase separations using gradient elution. AAS, inductively coupled plasma mass spectrometry (ICP-MS), and fluorometric detection can be used. HPLC coupled with AAS is commonly used for speciation of organotin compounds (Takeuchi et al. 2000).

7.1 BIOLOGICAL MATERIALS

Tin and its compounds can enter the human body through inhalation, ingestion, or penetration through the skin. Levels of tin and tin compounds in the body can be estimated by analysis of body fluids, excreta, or tissues. Methods for the determination of tin in biological materials are summarized in Table 7-1.

Normally, for determination in biological samples, the sample is digested in an oxidizing acid mixture followed by atomic spectrometric determination. Determination of organotin compounds in biological materials will require extraction, derivatization, separation, and detection, as described above. Human exposure to elemental tin and organotin compounds may be determined by analysis of blood or urine. Whole blood samples are typically analyzed by spectrophotometry and photometry. Urine samples may be acid digested to destroy organic matter and to oxidize tin to the tin(IV) state (Stewart and Lassiter 2001).

7.2 ENVIRONMENTAL SAMPLES

Methods for determination of tin in environmental samples are summarized in Table 7-2.

Tin is readily measured in multielement analyses of air, water, and solid waste samples by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). For samples that are free of particulate matter, such as drinking water, direct aspiration atomic absorption spectroscopy, such as EPA Method 7870, may be used. Other samples, such as groundwater, industrial wastes, soils, sediments, sludges, and other solid wastes, require digestion prior to analysis to determine total and acid leachable metal (EPA 1992). EPA Method 3050B, which describes acid digestion of sediments, sludges, and soils, does not list tin as an analyte; however, it states other elements and matrices may be analyzed by this method if performance is demonstrated for that analyte in that matrix at the concentrations of interest (EPA 1996b).

The APHA methods using either a flame atomic absorption method (3111B) or electrochemical atomic absorption method (3113B) may be used for analysis of tin in water, depending on the sensitivity desired.

Table 7-1. Analytical Methods for Determining Inorganic Tin and Organotin Compounds in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Total inorganic tin					
Biological material[a]	Digestion of biological materials	Atomic spectrometric	No data	No data	Angerer and Schaller 1988
Urine	Digest in oxidizing acid, extract ketone as the cupferon chelate	Colorimetry	<50 µg/L[b]	98–106%	Baselt 1988
Urine	Extraction with poly-dithiocarbamate resin, which is ashed	ICP-AES	2 µg/L	100±10% recovery	Kneip and Crable 1988
Urine	Extract with resin, ash resin	ICP-AES	0.1 µg	100±10%	NIOSH 1984a
Food	Digest in oxidizing acid	AAS	No data	No data	AOAC 1990a
Urine	Extract with resin, ash resin	ICP-AES	0.1 µg/sample	100%	NIOSH 1994a
Blood	Wet ashing with nitric and perchloric acids	AAS	2.5 ng/mL	No data	Chiba et al. 1994
Urine	Acidified with nitric acid	ICP-MS	0.05 µg/L	95.5%	Schramel et al. 1997
Organotins and metabolites					
Fruit	No data	Spectrophotometry (dithiol method)	0.2 µg	-98%	Corbin 1970
Biological materials, tissue	Homogenized, hydrochloric acid added, extracted with ethyl acetate	HPLC/fluorescence[c]	0.1–1 ng	91–100%	Yu and Arakawa 1983
Biological materials	Elution stepwise on silica gel column	AAS	1.5 ng	72.7±9.3%	Iwai et al. 1981
Human liver	Acidified tissue extracted with 0.1% tropolone-acetone, derivatization with propyl magnesium bromide	GC-FPD	5 ng/g (TBT) 1 ng/g (DBT, MBT)	No data	Kannan and Falandysz 1997)
Human liver	Homogenized with 0.1% tropolone-acetone/HCl, dervatized with propyl magnesium bromide	GC-FPD	4.0 ng/g (TBT) 3.0 ng/g (DBT) 2.0 ng/g (MBT)	No data	Takahashi et al. 1999

Table 7-1. Analytical Methods for Determining Inorganic Tin and Organotin Compounds in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human liver	Acid digestion, derivatization with sodium tetraethylborate	GC-PFPD	0.3 ng/g (TBT) 3 ng/g (DBT, MBT)	No data	Nielson and Strand 2002

[a]A digestion procedure for metals in biological materials applicable to most metals, including tin.
[b]Estimated from sensitivity and linearity data.
[c]Fluorescence detection after derivitization with Morin reagent.

AAS = atomic absorption spectroscopy; DBT = dibutyltin; FPD = flame photometric detector; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high performance liquid chromatography; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; ICP-MS = inductively coupled plasma mass spectrometryl; MBT = monobutyltin; PFPD = pulsed flame photometric detector; TBT = tributyltin

Table 7-2. Analytical Methods for Determining Inorganic Tin and Organotin Compounds in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Total inorganic tin					
Environmental	Digested in oxidizing acid	ICP-MS	0.04–50 ng/g	103±3%	Brzezinska-Paudyn and Van Loon 1988
Water	Generate hydride with sodium borohydride or electrolytically, sweep into silica cell heated to 700 °C	AAS	0.02 µg/L	No data	Rains 1982
Water (aqueous solution)	Generate hydride with sodium borohydride or electrolytically, sweep into silica cell heated to 700 °C	AAS	0.5 µg/L	No data	Thompson and Thomerson 1974
Water	Acidify with nitric acid	AAS (direct aspiration)	0.8 mg/L	No data	APHA 1998a
Water	Acidify with nitric acid	AAS (furnace technique)	5 µg/L	No data	APHA 1998b
Water[a]	Acidify with nitric acid	ICP-AES	No data	No data	APHA 1998c
Water	Acidify with nitric acid	AAS (direct aspiration)	0.8 mg/L	No data	EPA 1986b, 1992, 1996b
Pesticide formulations	Form volatile organotin derivatives	GC-FID	No data	No data	Basters et al. 1978
Organotins					
Pesticide formulations	Derivatize butylmagnesium chloride, extract with toluene	GC-FID	No data	No data	AOAC 1990a
Air	Adsorbed onto Chromosorb 102 desorption with ethereal hydrochloric acid, methylated	GC-FID	0.05 µg/m^3	93.3±9.3%	Zimmerli and Zimmermann 1980
Air[b]	Adsorption on filter and XAD-2 resin, desorption	HPLC-AAS (furnace technique)	1 µg/sample	No data	NIOSH 1994b
Air[c]	Adsorption on filter and XAS-2 resin, desorption, derivatization with sodium tetraethylborate	GC-FPD	0.01 µg	No data	NIOSH 2002

Table 7-2. Analytical Methods for Determining Inorganic Tin and Organotin Compounds in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Organotins (continued)					
Water	Acidified, extracted with tropolone benzene, dervatized	GC/FPD	100 pg	96±4 to 103±8%	Maguire and Huneault 1981
Water	Generate hydrides with sodium borohydride, separate hydrides by boiling point	AAS	2 ng	No data	Hodge et al. 1979
Water	Generate hydride derivatives	AAS	<0.1 µg/L tributyltin	No data	Lee et al. 1989
Water	Extract in n-hexane, produce fluorescent morin derivative	Fluorescence	0.001–0.5 nmol/mL	91.3±0.6 to 99.7±0.5% recovery	Arakawa et al. 1983

[a]Tin not listed specifically as an analyte, but can be determined by ICP-AES.
[b]Method was validated with tetrabutyltin, tributyltin chloride, tricyclohexylting hydroxide, and dibutyltin bis(isooctylmercaptoacetate).
[c]This method was developed for air monitoring of methyltin chlorides.

AAS = atomic absorption spectroscopy; GC-FID = gas chromatography-flame ignition detector; GC-FPD = gas chromatography-flame photometric detector; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; ICP-MS = inductively coupled plasma-mass spectrometry

While tin is not specifically listed as an analyte for the ICP-MS method (3125), it may also be used in most cases and has lower detection limits (APHA 1998a, 1998b, 1998c).

Organotin can be extracted from environmental samples and determined by AAS or GC methods, usually after derivatization and separation. NIOSH Method 5504 allows for analysis of organotin compounds (as tin) in air and was validated using tetrabutyltin, tributyltin chloride, tricyclohexylting hydroxide, and dibutyltin bis(isooctylmercaptoacetate) (NIOSH 1994b). NIOSH Method 5526 was developed for air monitoring of monomethyltin trichloride, dimethyltin dichloride, and trimethyltin chloride (NIOSH 2002).

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of tin and its compounds is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of tin and its compounds.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Sensitive and selective methods are available for the detection and quantitative measurement of tin after the sample matrix in which it is contained has been properly treated. Atomic spectrometric techniques provide methods for the determination of tin with low detection limits that are highly specific are readily available (Angerer and Schaller 1988; AOAC 1984b; Kneip and Crable 1988; NIOSH 1984a). Methods for the determination of specific compounds that contain tin are more difficult and less well developed than are methods for the

determination of total tin, but determination of specific tin compounds is an important concern because of the widespread use of organotin compounds as preservatives in industry and in other applications.

Exposure. Methods exist to determine inorganic and organic tin levels in environmental samples and human tissues. However, no methods have been identified that can be used to correlate the level and extent of exposure to tin and specific tin compounds with levels of tin in biological materials such as human tissues or fluids. It would be useful to have such methods to make these correlations; however, it is not likely that such a method will be developed.

Effect. No methods have been identified that can be used to directly associate levels of tin and specific tin compounds in biological samples with the onset of adverse health effects. If such methods were available, it would be possible to correlate the level or severity of effects with the level and extent of exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining tin in water, air, and waste samples with excellent selectivity and sensitivity are well developed and undergoing constant improvement.

Sampling methodologies for very low level elemental pollutants such as tin continue to pose problems, including nonrepresentative samples, insufficient sample volumes, contamination, and labor-intensive, tedious extraction, and purification procedures (Green and LePape 1987).

7.3.2 Ongoing Studies

No ongoing studies involving analytical techniques of tin or tin compounds were found in a search of Federal Research in Progress (FEDRIP 2003).